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(54) Title: RECOMBINANT C140 RECEPTOR AND ITS AGONISTS AND ANTAGONISTS

(57) Abstract

The DNA encoding the C140 cell surface receptor has been cloned and sequenced. The availability of this DNA permits the recombinant production of the C140 receptor which can be produced at cell or oocyte surfaces and is useful in assay systems both for the detection of substances which affect its activity, including agonists and antagonists. Further, the elucidation of the structure of the C140 receptor permits the design of agonist and antagonist compounds which are useful in these assays. The availability of the C140 receptor also permits production of antibodies specifically immunoreactive with the receptor per se or with specific regions thereof.

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RECOMBINANT C140 RECEPTOR AND ITS AGONISTS AND ANTAGONISTS

Technical Field

The invention relates to a newly discovered receptor which is a member of the G-protein-coupled receptor superfamily. The receptor is expressed in endothelial cells in blood vessels. Avoidance of effects on this receptor is an essential element in limiting side effects of drugs which are administered to stimulate other receptors in this family.

Background Art

Responses of animals to many therapeutic and prophylactic drugs are mediated through receptors which reside on cell surfaces. One class of such receptors comprises the G-protein-coupled receptors, whose physiological effect is mediated by a three-subunit protein complex, called G-proteins, that binds to this type of receptor with the subsequent release of a subunit, thus setting in motion additional intracellular events. Receptors of this subclass include, among others, adrenergic receptors, neuropeptide receptors, the thrombin receptor and the C140 receptor which is the subject of the herein invention. This class of receptor is characterized by the presence of seven transmembrane regions which anchor the receptor within the cell surface.

It is the elusive goal of the designers of therapeutic substances to effect a desired response in a subject in the absence of side effects. Accordingly, pharmaceuticals designed to target a specific receptor, such as the thrombin receptor, should react with the thrombin receptor specifically and have no effect on related receptors. The C140 receptor of the present

invention may be involved in controlling vascular pressure, and inadvertent stimulation or blocking of this receptor would have unpredictable and therefore undesirable results. It is therefore useful to determine in advance whether therapeutic reagents designed to target, for example, the thrombin receptor will or will not have the undesired side effect of reactivity with the C140 receptor. By providing the recombinant materials for the production of the C140 receptor in convenient assay systems, as well as agonist and antagonist reagents for use in this assay, the invention makes possible the prior determination of the presence or absence of the side effect of reactivity with the C140 receptor in candidate pharmaceuticals. This side effect will usually be undesired as it is believed that the C140 receptor responds to enzymes such as serine proteases associated with trauma and immune disturbances.

Disclosure of the Invention

The invention provides methods and materials useful in assay systems to determine the propensity of candidate pharmaceuticals to exert undesirable side effects. The isolation, recombinant production and characterization of the C140 receptor permits the design of assay systems using the receptor as a substrate and using agonists and antagonists for the receptor as control reagents in the assay.

Thus, in one aspect, the invention is directed to recombinant materials associated with the production of C140 receptor. These include, for example, transfected cells which can be cultured so as to display the C140 receptor on their surfaces, and thus provide an assay system for the interaction of materials with the native C140 receptor. In general, the limitations on the host cells useful in these assay systems are that the cells have the appropriate mechanism to display the receptor on

their surfaces and contain the G-protein as mediator to the intracellular response. (However assays which merely assess binding do not require the G-protein.) Most animal cells meet these requirements.

In another aspect, the invention is directed to C140 receptor agonists which mimic the activated form of the extracellular portion of the receptor protein. These agonists are useful as control reagents in the above-mentioned assays to verify the workability of the assay system. In addition, agonists for the C140 receptor may exhibit hypotensive effects *in vivo*. Accordingly, the agonists may be also, themselves, useful as antihypertensives.

In still another aspect, the invention is directed to C140 receptor antagonists. These antagonists comprise modified forms of the C140 receptor agonist peptides that lack the essential features required for activation of the receptor. These antagonists bind to receptor, do not activate it, and prevent receptor activation by agonists and the native receptor-binding ligand.

A second group of antagonists includes antibodies designed to bind specific portions of the receptor protein. In general, these are monoclonal antibody preparations which are highly specific for any desired region of the C140 receptor. The antibodies of the invention are also useful in immunoassays for the receptor protein, for example, in assessing successful expression of the gene in recombinant systems.

In another aspect, the invention is related to assay systems which utilize recombinant C140 receptor to screen for agonist and antagonist activity of candidate drugs. This assay is especially useful in assuring that these therapeutic agents do not have undesired side effects caused by activation or inhibition of the C140 receptor. In some cases agonist activity at this receptor system may have therapeutic utility. Some of these assay

systems include the use of the agonist peptides as positive controls. The assay can also be used to screen for antagonists which inhibit the agonistic effect.

Another aspect of the invention relates to the diagnosis of conditions characterized by activation of the C140 receptor by detection in fluids, such as blood or urine, of the peptide cleaved from the C140 receptor when the receptor is activated. Another diagnostic method included in the invention is visualization of the activated forms of receptor by localizing an imaging agent to activated receptor *in situ* using antibodies specific to the activated receptor.

Additional aspects of the invention are directed to pharmaceutical compositions containing the agonists and antagonists of the invention. The agonists of the invention are antihypertensives; conversely, the antagonists can elevate blood pressure if desired.

Brief Description of the Drawings

Figure 1 shows the DNA and deduced amino acid sequence of murine C140 receptor.

Figure 2 shows the DNA and deduced amino acid sequence of human C140 receptor.

Figure 3 shows a comparison of amino acid sequences for the human C140 receptor and murine C140 receptor.

Figure 4 shows a proposed model of C140 receptor activation based on the deduced amino acid sequence.

Figure 5 shows a comparison of amino acid sequences for the mouse C140 receptor and the human thrombin receptor.

Figure 6 shows the results of Northern Blot to detect the presence of mRNA encoding C140 receptor in various mouse tissues.

Figure 7 shows a trace of blood pressure demonstrating the *in vivo* hypotensive effect of a C140 agonist peptide.

Figure 8 shows blood vessel dilation in rat femoral vein induced by a C140 receptor agonist peptide. Figure 8a shows these results in the immobilized vein; Figure 8b shows these results for the immobilized vein depleted of endothelial cells.

Figure 9 shows the results of an assay for activation of the C140 receptor, expressed in frog oocytes, by plasmin, kallikrein, or trypsin. Figure 9a shows the results for plasmin; Figure 9b shows the results for kallikrein; Figure 9c shows the results for trypsin.

Figure 10 shows the nucleotide sequence and deduced amino acid sequence of a cDNA clone encoding murine C140 receptor.

Figure 11 shows the nucleotide sequence and deduced amino acid sequence of a cDNA clone encoding human C140 receptor.

Figure 12 shows the results of *in situ* hybridization of a sectioned newborn mouse with mouse C140 receptor probes.

Figure 13 shows a Northern blot of total RNA from human cell lines hybridized to a human C140 receptor probe.

Modes of Carrying Out the Invention

The characteristics of the C140 receptor elucidated by the invention herein are summarized in Figures 1-4. Figure 1 shows the complete DNA sequence of the clone encoding the murine receptor, along with the deduced amino acid sequence. As used herein, the "C140 receptor" refers to receptor in any animal species corresponding to the murine receptor contained in clone C140 described in Example 1 herein. Using the native DNA encoding the murine form of this receptor, the corresponding receptors in other species, including humans, as illustrated herein, may be obtained. Figure 2 shows the

corresponding DNA and deduced amino acid sequence of the human receptor.

The entire amino acid sequence of the murine receptor contains 395 amino acids, including a 27 amino acid signal peptide which, when cleaved, results in a 368 amino acid mature receptor protein. Similarly, the human receptor is encoded by an open reading frame corresponding to 398 amino acids including a probable 29 amino acid signal peptide sequence resulting in a 369 amino acid mature receptor protein, as shown in Figure 2.

Figure 3 shows a comparison of the human and murine amino acid sequences; as shown, these sequences exhibit a high degree of homology.

Hydrophobicity/hydrophilicity plots of the sequences shown in Figures 1 and 2 indicate that the mature C140 receptor is a member of the 7-transmembrane domain receptor family whose effect on the cell is mediated by G-protein. The mature C140 receptor has a relatively long extracellular amino acid extension containing several consensus sites for asparagine-linked glycosylation. It also contains a conserved asparagine in the first transmembrane region, the motif Leu-Ala-X-X-Asp in the second transmembrane region, a Trp in the fourth transmembrane region and a carboxy terminal tail which contains multiple serine and threonine residues. A proposed model of the *in situ* receptor is shown in Figure 4.

Referring to Figure 5, similarities to the thrombin receptor are readily seen. Figure 5 compares the amino acid sequence of murine C140 with that of thrombin receptor. It is known that the thrombin receptor is activated by proteolytic cleavage of the Arg-Ser bond at positions 41 and 42, which releases an activation peptide that permits refolding of the receptor and activation via the newly created amino terminus. In an analogous manner, the C140 receptor is activated by cleavage of the

Arg-Ser bond at positions 34 and 35, also liberating an activation peptide extending from position 1 of the putative mature protein to the cleavage site. It is believed that Arg-28 is the amino terminal amino acid residue of the mature protein, so the activation peptide has the sequence RNNSKGR. This peptide could thus be used as an index for activation of C140 receptor. In any event, the precise location of the N-terminus of the mature protein is unimportant for the design of agonists or antagonists. The activation peptide is likely to be freely filtered by the kidney and possibly concentrated in the urine and can be used as an index to activation of the C140 receptor.

Release of the activation peptide permits refolding of the receptor protein to activate the receptor. This is shown schematically in Figure 4, which also shows that the conformational changes resulting from the liberation of the activation peptide and refolding results in an intracellular conformational change of the receptor. This hypothesis is confirmed by the finding that the C140 receptor can be activated by a peptide mimicking the new amino terminus created by the activation. Accordingly, mimics of the N-terminus of the new amino terminus on the activated receptor behave as agonists therefor. The importance of the first five amino acids in the newly created amino terminus in the receptor for receptor activation has also been confirmed hereinbelow.

Based on this information, and by analogy with the mechanisms underlying trypsinogen activation to trypsin and activation of the thrombin receptor, it appears that the positively charged amino group on serine that is newly exposed when the ligand cleaves the receptor plays an important role in receptor activation. Peptides based on the agonist peptide sequence that bind the C140 receptor, but which are modified to be lacking the free α-amino group can function as antagonists of this

receptor. Thus, modifications of the agonist peptides which lack the capacity for specific activating interaction serve as C140 receptor antagonists.

Compounds of the Invention

The nomenclature used to describe the peptide compounds of the invention follows the conventional practice where the N-terminal amino group is assumed to be to the left and the carboxy group to the right of each amino acid residue in the peptide. In the formulas representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although often not specifically shown, will be understood to be in the form they would assume at physiological pH values, unless otherwise specified. Thus, the N-terminal H^+_2 and C-terminal O^- at physiological pH are understood to be present though not necessarily specified and shown, either in specific examples or in generic formulas. Free functional groups on the side chains of the amino acid residues can also be modified by amidation, acylation or other substitution, which can, for example, change the solubility of the compounds without affecting their activity.

In the peptides shown, each gene-encoded residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the amino acid, in accordance with the following conventional list:

Amino Acid	One-Letter Symbol	Three-letter Symbol
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

The amino acids not encoded genetically are abbreviated as indicated in the discussion below.

In the specific peptides shown in the present application, the L-form of any amino acid residue having an optical isomer is intended unless the D-form is expressly indicated by a dagger superscript (†).

The compounds of the invention are peptides which are partially defined in terms of amino acid residues of designated classes. Amino acid residues can be generally subclassified into four major subclasses as follows:

Acidic: The residue has a negative charge due to loss of H ion at physiological pH and the residue

is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

Basic: The residue has a positive charge due to association with H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

Neutral/nonpolar: The residues are not charged at physiological pH and the residue is repelled by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. These residues are also designated "hydrophobic" herein.

Neutral/polar: The residues are not charged at physiological pH, but the residue is attracted by aqueous solution so as to seek the outer positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium.

It is understood, of course, that in a statistical collection of individual residue molecules some molecules will be charged, and some not, and there will be an attraction for or repulsion from an aqueous medium to a greater or lesser extent. To fit the definition of "charged," a significant percentage (at least approximately 25%) of the individual molecules are charged at physiological pH. The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary and, therefore, amino acids specifically contemplated by the invention have been classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behavior.

Amino acid residues can be further subclassified as cyclic or noncyclic, and aromatic or nonaromatic, self-explanatory classifications with respect to the side chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of 4 carbon atoms or less, inclusive of the carboxyl carbon. Small residues are, of course, always nonaromatic.

For the naturally occurring protein amino acids, subclassification according to the foregoing scheme is as follows.

Acidic: Aspartic acid and Glutamic acid;

Basic/noncyclic: Arginine, Lysine;

Basic/cyclic: Histidine;

Neutral/polar/small: Glycine, serine, cysteine;

Neutral/nonpolar/small: Alanine;

Neutral/polar/large/nonaromatic: Threonine, Asparagine, Glutamine;

Neutral/polar/large aromatic: Tyrosine;

Neutral/nonpolar/large/nonaromatic: Valine, Isoleucine, Leucine, Methionine;

Neutral/nonpolar/large/aromatic: Phenylalanine, and Tryptophan.

The gene-encoded secondary amino acid proline, although technically within the group

neutral/nonpolar/large/ cyclic and nonaromatic, is a special case due to its known effects on the secondary conformation of peptide chains, and is not, therefore, included in this defined group.

Certain commonly encountered amino acids, which are not encoded by the genetic code, include, for example, beta-alanine (beta-Ala), or other omega-amino acids, such as 3-amino propionic, 2,3-diamino propionic (2,3-diaP), 4-amino butyric and so forth, alpha-aminisobutyric acid (Aib), sarcosine (Sar), ornithine (Orn), citrulline (Cit), t-butylalanine (t-BuA), t-butylglycine (t-BuG), N-methylisoleucine (N-MeIle), phenylglycine (Phg), and cyclohexylalanine (Cha), norleucine (Nle), cysteic acid (Cya) 2-naphthylalanine (2-Nal); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); β-2-thienylalanine (Thi); and methionine sulfoxide (MSO). These also fall conveniently into particular categories.

Based on the above definitions,

Sar, beta-Ala, 2,3-diaP and Aib are neutral/nonpolar/ small;

t-BuA, t-BuG, N-MeIle, Nle, Mvl and Cha are neutral/nonpolar/large/nonaromatic;

Orn is basic/noncyclic;

Cya is acidic;

Cit, Acetyl Lys, and MSO are neutral/polar/large/nonaromatic; and

Phg, Nal, Thi and Tic are neutral/nonpolar/large/ aromatic.

The various omega-amino acids are classified according to size as neutral/nonpolar/small (beta-Ala, i.e., 3-aminopropionic, 4-aminobutyric) or large (all others).

Other amino acid substitutions of those encoded in the gene can also be included in peptide compounds within the scope of the invention and can be

classified within this general scheme according to their structure.

All of the compounds of the invention, when an amino acid forms the C-terminus, may be in the form of the pharmaceutically acceptable salts or esters. Salts may be, for example, Na^+, K^+, Ca^{+2}, Mg^{+2} and the like; the esters are generally those of alcohols of 1-6C.

In all of the peptides of the invention, one or more amide linkages (-CO-NH-) may optionally be replaced with another linkage which is an isostere such as $-CH_2NH-$, $-CH_2S-$, $-CH_2CH_2-$, -CH=CH- (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2SO-$. This replacement can be made by methods known in the art. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Spatola, A.F., in "Chemistry and Biochemistry of Amino Acids Peptides and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983) (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general review); Hudson, D., et al., Int J Pept Prot Res (1979) 14:177-185 ($-CH_2NH-$, $-CH_2CH_2-$); Spatola, A.F., et al., Life Sci (1986) 38:1243-1249 ($-CH_2-S$); Hann, M.M., J Chem Soc Perkin Trans I (1982) 307-314 (-CH-CH-, cis and trans); Almquist, R.G., et al., J Med Chem (1980) 23:1392-1398 ($-COCH_2-$); Jennings-White, C., et al., Tetrahedron Lett (1982) 23:2533 ($-COCH_2-$); Szelke, M., et al., European Application EP 45665 (1982) CA:97:39405 (1982) ($-CH(OH)CH_2-$); Holladay, M.W., et al., Tetrahedron Lett (1983) 24:4401-4404 ($-C(OH)CH_2-$); and Hruby, V.J., Life Sci (1982) 31:189-199 ($-CH_2-S-$).

A. Agonists

The agonists of the invention comprise a series of peptides of the formula

$$AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6\text{-}AA_7\text{-}Z \quad (1)$$

wherein AA_1 is a small amino acid or threonine;

AA_2 and AA_3 are each independently neutral/nonpolar/large/nonaromatic amino acids;

AA_4 is a small amino acid;

AA_5 is a basic amino acid;

AA_6 may be present or absent and, if present, is a neutral/nonpolar/large/nonaromatic amino acid;

AA_7 is absent if AA_6 is absent and may be present or absent if AA_6 is present, and is an acidic amino acid; and

Z is a substituent that does not interfere with agonist activity.

The peptide of formula 1 can be extended (shown as included in Z) at the C-terminus (but not the N-terminus) by further amino acid sequence to comprise a noninterfering substituent.

At the C-terminus of the compounds of formula 1, the carboxyl group may be in the underivatized form or may be amidated or may be an ester; in the underivatized form the carboxyl may be as a free acid or a salt, preferably a pharmaceutically acceptable salt.

If the C-terminus is amidated, the nitrogen atom of the amido group, covalently bound to the carbonyl carbon at the C-terminus, will be NR'R', wherein each R' is independently hydrogen or is a straight or branched chain alkyl of 1-6C, such alkyls are 1-6C straight- or branched-chain saturated hydrocarbyl residues, such as methyl, ethyl, isopentyl, n-hexyl, and the like. Representatives of such amido groups are: $-NH_2$, $-NHCH_3$,

$-N(CH_3)_2$, $-NHCH_2CH_3$, $-NHCH_2CH(CH_3)_2$, and $-NHCH_2CH(CH_3)CH_2CH_3$, among others. Furthermore, either or both R' may in turn optionally be substituted by one or more substituents such as, for example, -OR', -NR'R', halo, -NR'CNR'NR'R' and the like, wherein each R' is as independently defined above. Thus, Z may be -OH, or an ester (OR') or salt forms thereof, or -NR'R' wherein R' is as above defined.

Preferred embodiments of AA_1 are Ser on 2,3-diaminopropionyl (2,3-diaP). Preferred embodiments of AA_2 and AA_3 are Val, Ile, Cha and Leu. Preferred embodiments for the residues in the remainder of the compound of formula (1) are those wherein AA_4 is Gly, AA_5 is Lys, Arg or Har, AA_6, if present, is Val, Ile, Cha or Leu, and AA_7, if present, is Asp or Glu. Particularly preferred are compounds of formula (1) which are selected from the group consisting of SLIGRLETQPPIT, SLIGRLETQPPI, SLIGRLETQPP, SLIGRLETQP, SLIGRLETQ, SLIGRLET, SLIGRLE, SLIGRL, SLIGR, SLLGKVDGTSHVT, SLLGKVDGTSHV, SLLGKVDGTSH, SLLGKVDGTS, SLLGKVDGT, SLLGKVDG, SLLGKVD, SLLGKV, SLLGK, S(Cha)IGR, S(Cha)LGK, (2,3-diaP)-IGR, (2,3-diaP)LLGK, SLLGKR-NH_2, SLIGRR-NH_2, S(Cha)LGKK-NH_2, S(Cha)IGRK-NH_2, (2,3-diaP)-LIGRK-NH_2, (2,3-diaP)-LLGKK-NH_2 and the amidated forms thereof.

B. Antagonists

Compounds of the invention which interfere with activities mediated by the C140 receptor include modified agonist peptides lacking the N-terminal serine residue; and antibodies which are immunoreactive with various critical positions on the C140 receptor.

Peptide Antagonists

The antagonists of the first group--modified agonists--can be represented by the formula:

$$X\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6\text{-}AA_7\text{-}Z$$

wherein X is an amino acid residue other than ser, ala, thr, cys, 2,3-diaP or gly or is a desamino or alkylated or acylated amino acid,

wherein AA_2 and AA_3 are each independently neutral/nonpolar/large/nonaromatic amino acids;

AA_4 is a small amino acid;

AA_5 is a basic amino acid;

AA_6 may be present or absent and, if present, is a neutral/nonpolar/large/nonaromatic amino acid;

AA_7 is absent if AA_6 is absent and may be present or absent if AA_6 is present, and is an acidic amino acid; and

Z is a substituent that does not interfere with agonist activity.

Preferred acyl groups are of the formula RCO- wherein R represents a straight or branched chain alkyl of 1-6C. Acetyl is particularly preferred.

Preferred embodiments of X include residues of 3-mercaptopropionic acid (Mpr), 3-mercaptovaleric acid (Mvl), 2-mercaptobenzoic acid (Mba) and S-methyl-3-mercaptopropionic acid (SMeMpr). Preferred embodiments for AA_2 through AA_7 are as described for the agonists above; Z is also as thus described.

Particularly preferred among the antagonist peptides of this class are those selected from the group consisting of Mpr-LLGK, Mpr-LIGR, Mpr-(Cha)LKG, Mpr-(Cha)IGR, Mpr-LLGKK-NH_2, Mpr-LIGRK-NH_2, Mpr-LIGRKETQP-NH_2, Mpr-LLGKKDGTS-NH_2, $(n\text{-pentyl})_2$-N-Leu-Ile-Gly-Arg-Lys-NH_2 and (Me-N-(n-pentyl)-Leu-Ile-Gly-Arg-Lys-NH_2.

Antibodies

Antagonists which are antibodies immunoreactive with critical positions of the C140 receptor are obtained by immunization of suitable mammalian subjects with peptides containing as antigenic regions those portions of the C140 receptor intended to

be targeted by the antibodies. Critical regions include the region of proteolytic cleavage, the segment of the extracellular segment critical for activation (this includes the cleavage site), and the portions of the sequence which form the extracellular loops, in particular, that region which interacts with the N-terminus of the activated receptor extracellular region. The agonist peptides of the invention may be used as immunogens in this case.

Thus, peptides which contain the proteolytic region, namely, for example, SKGRSLIGRLET, the extracellular loops, such as those including ISY HLHGNNWVYGEALC; QTIYIPALNITTCHDVLPEEVLVGDMFNYFL; and HYFLIKTQRQSHVYA. The agonist peptides described below are also useful as immunogens.

The antibodies are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptide haptens alone, if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten or is the C140 receptor itself displayed on a recombinant host cell. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either in vitro or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of receptor can also be produced in the context of chimeras with multiple species origin.

The antibodies thus produced are useful not only as potential antagonists for the receptor, filling the role of antagonist in the assays of the invention, but are also useful in immunoassays for detecting the activated receptor. As such these antibodies can be coupled to imaging agents for administration to a subject

to allow detection of localized antibody to ascertain the position of C140 receptors in either activated or unactivated form. In addition, these reagents are useful in vitro to detect, for example, the successful production of the C140 receptor deployed at the surface of the recombinant host cells.

Preparation of Peptide Agonists and Antagonists

The peptide agonists and antagonists of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Recombinant Production of C140 Receptor for Use in Assays

The invention provides recombinant materials for the production of C140 receptor for display on the surface of recombinant cells. Production of the receptor using these recombinant methods provides a useful reagent to determine the ability of a candidate drug to bind to, to activate, or to antagonize the C140 receptor. Determination of these properties is essential in evaluating the specificity of drugs intended for binding other related receptors.

For this recombinant production, a DNA sequence encoding the C140 receptor, such as those set forth in Figures 1 and 2, or their substantial equivalents or their degenerate analogs, is prepared either by retrieval of the native sequence, as set forth below, or by using substantial portions of the known native sequence as probe, or can be synthesized de novo

using standard procedures. The DNA is ligated into expression vectors suitable for the desired host and transformed into compatible cells. The cells are cultured under conditions which favor the expression of the C140 receptor encoding gene and the cells displaying the receptor on the surface are harvested for use in the assays.

The host cells are typically animal cells, most typically mammalian cells. In order to be useful in the assays, the cells must have intracellular mechanisms which permit the receptor to be displayed on the cell surface in the configuration shown generally in Figure 4 herein. If the assay uses cellular response to activated receptor as a detection system, the cells must also contain a G-protein linked mechanism for response to activation of the receptors. Most mammalian and other animal cells fulfill these qualifications.

Particularly useful cells for use in the method of the invention are *Xenopus laevis* frog oocytes, which typically utilize cRNA rather than standard recombinant expression systems proceeding from the DNA encoding the desired protein. Capped RNA is typically produced from linearlized vectors containing DNA sequences encoding the receptor. The reaction is conducted using RNA polymerase and standard reagents. cRNA is recovered, typically using phenol/chloroform precipitation with ethanol and injected into the oocytes.

The animal host cells expressing the DNA encoding the C140 receptor or the cRNA-injected oocytes are then cultured to effect the expression of the encoding nucleic acids so as to produce the C140 receptor displayed in a manner analogous to that shown in Figure 4 on their surfaces. These cells then are used directly in assays for assessment of a candidate drug to bind, antagonize, or activate the receptor.

Assays

In one type of easily conducted assay, competition of the candidate drug for binding to the receptor with either agonist or known binding antagonist can be tested. In one method, the competing agonist or antagonist may be labeled; the labeled substance known to bind the receptor can, of course, be a synthetic peptide. In one typical protocol, varying concentrations of the candidate are supplied along with a constant concentration of labeled agonist or antagonist and the inhibition of a binding of label to the receptor can be evaluated using known techniques.

In a somewhat more sophisticated approach, the effect of candidate compounds on agonist-induced responses can be measured in the cells recombinantly expressing the C140 receptor as described below. Assay systems for the effect of activation of receptor on these cells include calcium mobilization and voltage clamp which are described herein in further detail. These assays permit an assessment of the effect of the candidate drug on the receptor activity rather than simply ability to bind to the receptor.

Agonist-induced increases in ^{45}Ca release by oocytes expressing cRNA encoding C140 receptor or other recombinant cells producing C140 receptor are assessed by published techniques (Williams, J.A., et al., Proc Natl Acad Sci USA (1988) 85:4939-4943). Briefly, intracellular calcium pools are labeled by incubating groups of 30 oocytes in 300 μl calcium-free modified Barth's solution (MBSH) containing 50 μCi $^{45}CaCl_2$ (10-40 mCi/mg Ca; Amersham) for 4 hours at RT. The labeled oocytes or cells are washed, then incubated in MBSH II without antibiotics for 90 minutes. Groups of 5 oocytes are selected and placed in individual wells in a 24-well tissue culture plate (Falcon 3047) containing 0.5 ml/well MBSH II without antibiotics. This medium is removed and

replaced with fresh medium every 10 minutes; the harvested medium is analyzed by scintillation counting to determine ^{45}Ca released by the oocytes during each 10-minute incubation. The 10-minute incubations are continued until a stable baseline of ^{45}Ca release per unit time is achieved. Two additional 10-minute collections are obtained, then test medium including agonist is added and agonist-induced ^{45}Ca release determined.

Using the above assay, the ability of a candidate drug to activate the receptor can be tested directly. In this case, the agonists of the invention are used as controls. In addition, by using the agonist of the invention to activate the recombinant receptor, the effect of the candidate drug on this activation can be tested directly. Recombinant cells expressing the nucleic acids encoding the receptor are incubated in the assay in the presence of agonist with and without the candidate compound. A diminution in activation in the presence of the candidate will indicate an antagonist effect. Conversely, the ability of a candidate drug to reverse the antagonist effects of an antagonist of the invention may also be tested.

In an alternative to measuring calcium mobilization, the voltage clamp assay can be used as a measure for receptor activation. Agonist-induced inward chloride currents are measured in voltage-clamped oocytes expressing C140 receptor encoding cRNA or cells expressing DNA from recombinant expressions systems essentially as previously described (Julius, D., et al, Science (1988) 241:558-563) except that the single electrode voltage-clamp technique is employed.

Detection of Activated Receptors

In one embodiment, the availability of the recombinant C140 receptor protein permits production of antibodies which are immunospecific to the activated form of the receptor which can then be used for diagnostic imaging of activated receptors in vivo. These antibodies are produced either to the activated form of the receptor produced recombinantly, or to the peptide representing the "new amino terminal" peptide described herein. The resulting antibodies, or the immunospecific fragments thereof, such as the Fab, Fab', Fab'_2 fragments are then conjugated to labels which are detected by known methods, such as radiolabels including technetium^{99} and indium^{111} or other radioactive labels as is known in the art. When injected in vivo, these antibodies home to the sites of activated receptor, thus permitting localization of areas containing activated receptors.

In another embodiment, the presence of the activation peptide in body fluids or in culture media can be detected and measured. Antibodies are made to the activation peptide as described above and can be employed in standard ELISA or RIA assays to detect excess amounts of the activation peptide in, for example, urine.

Administration of Agonists and Antagonists as Pharmaceuticals

The peptides of the invention which behave as agonists are administered in conventional formulations for systemic administration as is known in the art. Typical such formulations may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition.

Preferred forms of systemic administration of peptides include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can also be used.

More recently, alternative means for systemic administration of peptides have been devised which include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the patient's condition, and the judgment of the attending physician. Suitable dosage ranges, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of peptides available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art.

As shown hereinbelow, the agonists of the invention behave as antihypotensives; antagonists have the opposite effect. Thus, patients whose blood pressure needs to be raised or lowered benefit by the administration of the suitable peptide.

In addition, the agonists have anti-inflammatory and wound healing properties.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Isolation of the Gene Encoding Murine C140 Receptor

A mouse cosmid genomic library (obtained from Dr. R.A. Wetsel, Washington University School of Medicine, St. Louis, Missouri and described in Wetsel, R.A. *et al.*, J Biol Chem (1990) 265:2435-2440) was screened with two ^{32}P-labeled oligonucleotides corresponding to bp 190-249 and 742-801, respectively, of the bovine substance K receptor cDNA (Masu, Y. *et al.*, Nature (1987) 329:836-838). The hybridization conditions are 5 x SSC, 5 x Denhardt's, 0.1% SDS, 0.1 mg/ml sperm DNA, 10^6 cpm/ml of labeled oligonucleotides, 60°C overnight, followed by washing with 1 x SSC, 0.1% SDS at 60°C.

In one of the clones isolated (C140) the hybridizing region was localized to a 3.7 kb PstI fragment. This fragment was subcloned into the commercially available pBluescript vector. The hybridizing and adjacent regions were sequenced in both orientations by the Sanger chain termination method. Figure 1 shows both the nucleotide sequence and the deduced amino acid sequence of the mouse C140 receptor. The tentative signal sequence (SP) and the seven transmembrane regions are overlined, potential asparagine-linked glycosylation sites are marked with bold arrows, and the putative protease receptor cleavage site at Arg34-Ser35 is marked with an open arrow.

Example 2

Isolation of the Gene Encoding Human C140 Receptor

The availability of genomic DNA encoding the mouse protease C140 receptor permitted the retrieval of the corresponding human gene. A human genomic library cloned in the vector EMBL3 was screened at exactly the conditions in Example 1 using the entire coding region of the murine clone as a probe. The recovered human gene

including the DNA sequence and the deduced amino acid sequence are shown in Figure 2. Subsequent experiments indicated that the human C140 gene is located in the same region of the long arm of chromosome number 5 (5q12-5q13) as has been reported for the human thrombin receptor gene.

In addition, a 1.1 kb genomic DNA fragment was obtained from Genome Systems Inc., commercial screening service as was PCR-positive with a primer pair that generates a fragment spanning 350-nucleotides of the human C140 protein coding region. A 1.1 kb bamH1 fragment was subcloned and sequenced and found to contain 800-nucleotides of promoter sequence. The promoter lacks both a TATA box and a CAAT box but is rich in G's and C's; features common to promoters of many housekeeping genes. Two binding elements specific for SP1 and AP2 were identified.

Example 3

Comparison of Related G-Protein Receptors

As shown in Figure 3, the deduced amino acid sequence of the human protease C140 receptor shows extensive similarity (>90%) to the mouse sequence.

Figure 5 shows an amino acid sequence alignment between the mouse C140 receptor and the related G-protein receptor human thrombin receptor (Coughlin, S. Cell). The tentative signal sequences (SP), transmembrane regions, and protease cleavage sites are marked.

Example 4

Recovery of Mouse C140 cDNA

A cDNA library from a mouse stomach was constructed in λ gt10 and screened with a probe encompassing the C1040 genomic DNA. A single phage clone

was isolated and cut with EcoRI. The insert was cloned into pBluescript and pSG5 and sequenced.

The isolated cDNA was 2732 nucleotides long including a 16 base polyA-stretch; 5' RACE resulted in the addition of only 27 bases to the 5' end. The 5' end of the apparent coding region differs from the 5' end of the open reading frame of genomic DNA; it is believed that the 5' end of the cDNA is correct. The complete nucleotide sequence and deduced amino acid sequence of murine cDNA encoding C140 is shown in Figure 10.

Example 5

Recovery of Human cDNA Encoding C140

A human intestinal tumor cDNA library was subjected to PCR using primers designed from the genomic clone of Example 2 and the amplified fragment was cloned in pSG5 and sequenced. The nucleotide sequence and deduced amino acid sequence are shown in Figure 11. There are four amino acid differences between the cDNA encoded sequence and that encoded by the genomic DNA as is shown in Figure 11.

Example 6

Activation of Protease C140 Receptor in Oocytes

Both native and mutant C140 receptors were produced in oocytes and activated with a peptide mimicking the new amino-terminus", or by the proteolytic enzyme trypsin (which cleaves the extracellular region). Native receptors were produced by cloning the coding region of the receptor gene, using the polymerase chain reaction, into the expression vector pSG-5 (Green, S. *et al.*, Nucleic Acid Res (1988) 16:369). The orientation and integrity of the cloned coding region was verified by determining the nucleotide sequence with the Sanger chain-termination method. Site-directed mutagenesis was employed to construct mutant receptors in the pSG-5.

Three mutant receptors were made, in which serine-35 was replaced with proline, arginine, and histidine, respectively. The nucleotide sequences of the three mutants was verified as above.

In order to produce the receptor at the surface of oocytes, cRNA encoding the receptor was produced as follows. pSG-5 C140 plasmid DNA was made linear by digestion with XbaI, and capped cRNA was produced *in vitro* using T7 RNA polymerase (Krieg and Melton, Meth Enzymol (1987) 155:397-415.

Oocytes from Xenopus laevis were harvested and prepared using published techniques (Coleman, A., in Hames, B.D., and Higgins, S.J., eds, Transcription and Translation: A Practical Approach, IRL Press, pp. 271-302; Williams, J.A., *et al*. Proc Natl Acad Sci USA (1988) 85:4939-4943]. To remove follicular cells, oocytes were incubated for 1.5 h with shaking in calcium-free Barth's containing 2 mg/ml each of collagenase 1A and hyaluronidase 1S. The oocytes were then washed five times in regular Barth's and incubated at 18°C in Barth's medium containing 100 U/ml penicillin, 100μg/ml streptomycin, and 2.5 mM sodium pyruvate. Stage V oocytes were selected and injected with 30 nl of cRNA (0.33 μg/μl water) or water alone, and then incubated with 0.25 ml of medium in groups of four/well in a 96-well culture plate. After 36 hours the oocytes were incubated with ^{45}Ca (250 μCi/ml). After 12 h incubation the oocytes were washed and 0.2 ml of medium added and replaced every five minutes. The harvested medium was analyzed by scintillation counting. After five replacements to determine the baseline release of ^{45}Ca, test medium with the agonist, e.g. SLIGRL, was added and the evoked ^{45}Ca-release determined.

Oocytes were injected with capped cRNA (ca 10 ng) encoding wild-type mouse C140 receptor (WT) or either of the three mutant receptors 35Pro, 35Arg and

35His. After 36 hours, cRNA-injected and control water-injected, oocytes were loaded with ^{45}Ca, and 12 hours thereafter peptide or trypsin-induced ^{45}Ca release were determined as described above. The peptide SLIGRL was added at 100 μM, and trypsin at 300 pM. The stimulation with the peptide was done on the same group of oocytes after the stimulation with trypsin. The data shown in Table 1 represent the mean of three replicate determinations, and denotes the increase compared to oocytes injected with water.

Table 1

Receptor	Agonist	Fold increase in ^{45}Ca
WT	Trypsin	6.6
35Pro	Trypsin	0
35Arg	Trypsin	0
35His	Trypsin	0
WT	SLIGRL	11
35Pro	SLIGRL	23
35Arg	SLIGRL	15
35His	SLIGRL	23

As shown in Table 1, the agonist peptide SLIGRL was able to activate both the wild-type and mutated receptors. On the other hand, trypsin, which can activate only by cleavage of the extracellular domain, is able only to activate the wild-type receptor.

Example 7

Activation of the C140 Receptor by Different Agonist Peptides

Various peptides were tested at 100μM in the assay above using wild-type mouse C140 receptor, expressed in oocytes. The results are shown in Table 2.

Table 2

Peptide	Fold Increase in ^{45}Ca
SLIGRL	15
SLIGRA	8.5
SLIGAL	0
SLIARL	4.3
SLAGRL	0
SAIGRL	0
ALIGRL	1.3
SFFLRW	1.7

The "native" peptide SLIGRL is most effective; replacing L at position 6 with alanine lowers but does not destroy activity. Positions 2 and 3 are more sensitive. Position 1 tolerates substitution with alanine but decreases the activity by a factor of 10; the activity of this agonist is comparable to the analogous thrombin receptor agonist SFFLRW.

Example 8

Expression of C140 Receptor in Various Tissues

Poly(A)+RNA was prepared from mouse tissues, resolved on a 1.2% agarose gel containing 50% formamide and blotted onto Hybond C extra membrane (Amersham). The blot was hybridized with a ^{32}P-labeled "random priming probe" directed against the whole coding region of murine C140 receptor. The probe was hybridized at 42°C for 48 hr then successively washed at 20°C in 1 X SSC, 0.1% SDS twice, 5 min each time, then at 65°C in 1 X SSC, again twice for 20 min each time, and then 0.1 X SSC, 0.1% SDS twice for 20 min each time. The resulting membrane was autoradiographed for 5 days at -80°C with an intensifying screen.

The results, shown in Figure 6 indicate that kidney and small intestine, but not spleen, contain mRNA encoding C140. In Figure 6, where each lane contains 10 μg RNA, lane A is derived from spleen, lane B from kidney and lane C from small intestine.

Example 9

Expression of C140 Transcripts In Mice

In situ hybridization using ^{35}S RNA probes was used to localize C140 transcripts in mouse embryogenesis and in adult mouse tissues. A strong signal was found in the gastrointestinal tract at 11.5 days; at 14 days there was strong hybridization to epithelial structures in the nasopharynx, stomach-intestine, skin and endothelial cells in larger vessels. There was some hybridization in the liver and sclerotoma but no signal in muscle or CNS. At 17 days, the signals in the sclerotoma had disappeared and additional epithelial structures showed hybridization including the esophagus, kidney glomeruli, lung, hair follicles and epidermis.

In newborns, the signals found at 17 days were retained and additional signals were found in the thymic medulla and kidney medulla. Adults showed transcripts in the mucosa of stomach, intestine and colon, white pulp of the spleen, thymus and kidney medulla. Again, there were no signals in the CNS, liver, lung or adrenal gland. Figure 12 shows the results of *in situ* hybridization in a sectioned newborn mouse using these probes.

Example 10

Expression of C140 Transcripts In Human Tissues

Figure 13 shows the results of a Northern blot of total RNA from human cell lines hybridized to a human C140 receptor probe. Ten mg of total RNA was used. Hybridization was obtained in RNA from stomach (lane 1), Ca-Co-2 cells (lane 2); HT-29 cells (lane 3), A498 cells (lane 5), 5637 cells (lane 8); skin kdratinocytes (lane 12), and HUVEC (lanes 13 and 14). No hybridization was detected in HuTu80 cells, J82 cells, MCF-7, HeLa or NCI 12 cells (lanes 4, 6, 9 and 10).

Example 11

Determination of Hypotensive Activity of C140 Agonists

The C140 agonist SLIGRL was injected in 0.2 ml buffer at various concentrations into rat femoral vein and the arterial pressure was monitored. The results of various concentrations are shown in Figure 7.

The trace in Figure 7 shows that even at 0.1 mM an appreciable decrease in blood pressure occurred; larger decreases were observed at 1 mM concentration.

This effect was also shown by observing vasodilation as a result of stimulation of the rat femoral vein with the above agonist. Adult Sprague-Dawley rats were killed by exsanguination during diethylether anesthesia and the femoral vein was removed and dissected free from fat and connective tissue. Circular preparations of the vein were mounted in an organ bath (5 ml) on two L-formed metal holders (0.2 mm diameter). One of the metal holders was screwed into one of the levers of a Grass FTO C force displacement transducer. The bathing liquid was Kreb's Ringer solution containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 1.2 mM KH_2PO_4 and 5.6 mM glucose. The bathing fluid was continuously treated with 88.5% oxygen-11.5% CO_2; the temperature was held at 37°C. The endothelium was removed by bubbling CO_2 through the vessels. The basal tension was between 7.5 and 12 mN. The preparations were equilibrated for at least 1 hr before application of agonist and control substances.

The results of these determinations are shown in Figure 8a and 8b. As shown in Figure 8a, contraction induced by application of $PGF_{2\alpha}$ at 3 X 10^{-5} M is relaxed by administration of 10^{-5} M agonist. The results in Figure 8a were obtained using the vein with the endothelium still present.

In Figure 8b, the endothelium has been removed. In an analogous experiment, the contraction induced by 3 X 10^{-5} M $PGF_{2\alpha}$ is not counteracted by 10^{-5} M agonist or by 10^{-5} M acetylcholine.

Example 8

Activation of Recombinant C140 Receptor by Plasmin and Kallikrein

Figures 9a and 9b show the ability of plasmin and kallikrein respectively to activate oocytes injected with C140 cRNA (open circles) or water (crosses) as control. Figure 9c shows the ability of trypsin to activate frog oocytes injected with C140 receptor cRNA (filled circles) or substance K receptor cRNA (open circles). Trypsin clearly has a differential effect on the C140 receptor-injected oocytes.

CLAIMS

1. A DNA molecule comprising an expression system capable, when transformed into a recombinant host, of producing the C140 receptor at the cell surface of the host, which expression system comprises a nucleotide sequence encoding the C140 receptor operably linked to a control sequence heterologous to said encoding nucleotide and operable in said host cell.

2. A cell modified to contain the expression system of claim 1.

3. A method to produce cells that contain C140 receptor deployed at their surface, which method comprises culturing the cells of claim 2 under conditions which effect the expression of the nucleotide encoding the C140 receptor to obtain said cells that contain C140 receptor deployed at their surface.

4. A cRNA molecule that encodes the C140 receptor.

5. Cells which are oocytes modified to contain the cRNA of claim 4.

6. A method to produce cells which are oocytes that contain C140 receptor deployed at their surface, which method comprises culturing the oocytes of claim 5 under conditions which effect the expression of the cRNA encoding the C140 receptor to obtain said cells that contain C140 receptor deployed at their surface.

7. A method to determine the C140 agonist activity of a candidate substance, which method comprises:

incubating the cells of claim 3 or 6 in the presence and absence of the substance, and

detecting the presence, absence or amount of activation of the C140 receptor in the presence as compared to the absence of said substance whereby an increase in said activation in the presence as compared to the absence of said substance indicates agonist activity of the substance.

8. A method to assess the ability of a candidate substance to behave as a C140 antagonist, which method comprises:

incubating the cells of claim 3 or 6 in the presence of a C140 agonist and in the presence and absence of said candidate, and

measuring the activation of the C140 receptor in the presence and absence of said candidate, whereby a decrease in said activation in the presence of the candidate indicates the antagonist activity of the candidate.

9. A method to assess the ability of a candidate substance to bind to C140 receptor, which method comprises:

incubating the cells of claim 3 or 6 in the presence of a C140 agonist or a known C140 antagonist and in the presence and absence of said candidate, and

measuring the binding of said C140 agonist or C140 antagonist to the surface of said cells in the presence and absence of said candidate, whereby a decrease in said binding in the presence of the candidate indicates the ability of the candidate to bind receptor.

10. An antibody composition specifically immunoreactive with an extracellular region of the C140 receptor protein or a portion thereof.

11. The antibody composition of claim 10 wherein said region is the ligand-binding region, or

which is specifically immunoreactive with activated C140 receptor, or

recognizes an epitope within the receptor sequence SLIGRL, or

is specifically reactive with the cleaved activation peptide of the C140 receptor.

12. A method to localize activated C140 receptors *in situ*, which method comprises:

administering to a subject putatively harboring activated C140 receptor an amount of antibody specific to said activated receptor effective to bind to said activated receptor, and

detecting the location of said antibody.

13. A method for detecting the presence of activated C140 receptor in a mammalian subject, which method comprises:

contacting a sample of the biological fluid of said subject with a detection system which measures the presence, absence or amount of the cleaved activation peptide of the C140 receptor; and

detecting the presence, absence or amount of said cleaved peptide.

14. An agonist peptide capable of activating C140 receptor, which peptide is of the formula

$$AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6\text{-}AA_7\text{-}Z \quad (1)$$

wherein AA_1 is a small amino acid or threonine;

AA_2 and AA_3 are each independently neutral/nonpolar/large/nonaromatic amino acids;

AA_4 is a small amino acid;

AA_5 is a basic amino acid;

AA_6 may be present or absent and, if present, is a neutral/nonpolar/large/nonaromatic amino acid;

AA_7 is absent if AA_6 is absent and may be present or absent if AA_6 is present, and is an acidic amino acid; and

Z is a substituent that does not interfere with agonist activity.

15. The peptide of claim 14 wherein AA_1 is ser, ala, gly, thr, or 2,3-diamino-propionic (2,3-diaP); and/or

wherein each of AA_2 and AA_3 is independently selected from the group consisting of ile, val, leu, and Cha; and/or

wherein AA_4 is Gly; and/or

wherein AA_5 is Arg, Lys or Har; and/or

wherein Z comprises OR', or NR'R' wherein each R' is independently H or is a straight or branched chain alkyl or 1-6C, wherein each R' may optionally be substituted with one or more substituents selected from the group consisting of -OR', -NR'R', and -NR'CNR'NR'R' wherein each R' is H or is a straight or branched chain alkyl of 1-6C.

16. The peptide of claim 15 wherein AA_1-AA_2-AA_3 is selected from the group consisting of SLI, SLL, SChaI, SChaL, (2,3-diaP)LI and (2,3-diaP)LL; and/or

wherein Z includes additional peptide sequence of 1-5 amino acids.

17. The peptide of claim 14 which is selected from the group consisting of SLIGRLETQPPIT, SLIGRLETQPPI, SLIGRLETQPP, SLIGRLETQP, SLIGRLETQ, SLIGRLET, SLIGRLE, SLIGRL, SLIGR, SLLGKVDGTSHVT,

SLLGKVDGTSHV, SLLGKVDGTSH, SLLGKVDGTS, SLLGKVDGT, SLLGKVDG, SLLGKVD, SLLGKV, SLLGK, S(Cha)IGR, S(Cha)LGK, (2,3-diaP)-LIGR, (2,3-diaP)LLGK, SLLGKR-NH_2, SLIGRR-NH_2, S(Cha)LGKK-NH_2, S(Cha)IGRK-NH_2, (2,3-diaP)-LIGRK-NH_2, and (2,3-diaP)-LLGKK-NH_2.

18. A peptide capable of inhibiting the function of the C140 receptor which peptide is of the formula

$$X\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6\text{-}AA_7\text{-}Z$$

wherein X is an amino acid residue other than ser, ala, thr, cys, 2,3-diaP or gly or is a desamino or acylated amino acid,

wherein AA_2 and AA_3 are each independently neutral/nonpolar/large/nonaromatic amino acids;

AA_4 is a small amino acid;

AA_5 is a basic amino acid;

AA_6 may be present or absent and, if present, is a neutral/nonpolar/large/nonaromatic amino acid;

AA_7 is absent if AA_6 is absent and may be present or absent if AA_6 is present, and is an acidic amino acid; and

Z is a substituent that does not interfere with agonist activity.

19. The peptide of claim 18 wherein X is Mvl, Mpr, Mba, or SMeMpr; and/or

wherein each of AA_2 and AA_3 is independently selected from the group consisting of ile, val, leu, Nle, Nva, Cyclopentylalanine and Cha; and/or

wherein AA_4 is Gly; and/or

wherein AA_5 is Arg, Lys, Orn or Har; and/or

wherein Z comprises OH or an ester or salt thereof, or NR'R' wherein each R' is independently H or

is a straight or branched chain alkyl of 1-6C, wherein each R' may optionally be substituted with one or more substituents selected from the group consisting of -OR', -NR'R', and -NR'CNR'NR'R' wherein each R' is H or is a straight or branched chain alkyl of 1-6C.

20. The peptide of claim 19 wherein AA_2-AA_3 is selected from the group consisting of LI, LL, ChaI, and ChaL; and/or

wherein Z includes a peptide extension of 1-5 amino acid residues.

21. The peptide of claim 18 which is selected from the group consisting of Mpr-LLGK, Mpr-LIGR, Mpr-(Cha)LKG, Mpr-(Cha)IGR, Mpr-LLGKK-NH_2, Mpr-LIGRK-NH_2, Mpr-LIGRKETQP-NH_2, Mpr-LLGKKDGTS-NH_2, $(n\text{-pentyl})_2$-N-Leu-Ile-Gly-Arg-Lys-NH_2 and (Me-N-(n-pentyl)-Leu-Ile-Gly-Arg-Lys-NH_2, and the amidated or acylated forms thereof.

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                CCCTGTCAGTCTTAAGATTCTAGAAGTCGCTGTCCTATACGGAACCCAAAA
CTCTCACTGTTAATGAAATACCATTGTCGGGGCGAAGATGTAGCTCAGTGGTAAAATACT -121

TGCCAGCACACACAAGAATTAGACTTCAACCGTCACCAACTGCCCTGTGTAGGACGGTCG
GTCACTGAAAGAGAATATTGTCTGCAATACTCTAATGACATCTGTCTGTGTTCATCTGAA -1

                                        SP
  1 MetPheHisLeuLysHisSerSerLeuThrValGlyProPheIleSerValMetIleLeu
    ATGTTCCATTTAAAACACAGCAGCCTTACTGTTGGACCATTTATCTCAGTAATGATTCTG

                          ▼                 ▽
    LeuArgPheLeuCysThrGlyArgAsnAsnSerLysGlyArgSerLeuIleGlyArgLeu
    CTCCGCTTTCTTTGTACAGGACGCAACAACAGTAAAGGAAGAAGTCTTATTGGCAGATTA 120

 41 GluThrGlnProProIleThrGlyLysGlyValProValGluProGlyPheSerIleAsp
    GAAACCCAGCCTCCAATCACTGGGAAAGGGGTTCCGGTAGAACCAGGCTTTTCCATCGAT

                                                          ______
    GluPheSerAlaSerIleLeuThrGlyLysLeuThrThrValPheLeuProValValTyr
    GAGTTCTCTGCGTCCATCCTCACCGGGAAGCTGACCACGGTCTTTCTTCCGGTCGTCTAC 240

                          ____________ I ____________
 81 IleIleValPheValIleGlyLeuProSerAsnGlyMetAlaLeuTrpIlePheLeuPhe
    ATTATTGTGTTTGTGATTGGTTTGCCCAGTAATGGCATGGCCCTCTGGATCTTCCTTTTC

                          ____________ II ____________
    ArgThrLysLysLysHisProAlaValIleTyrMetAlaAsnLeuAlaLeuAlaAspLeu
    CGAACGAAGAAGAAACACCCCGCCGTGATTTACATGGCCAACCTGGCCTTGGCCGACCTC 360

                ____________
121 LeuSerValIleTrpPheProLeuLysIleSerTyrHisLeuHisGlyAsnAsnTrpVal
    CTCTCTGTCATCTGGTTCCCCCTGAAGATCTCCTACCACCTACATGGCAACAACTGGGTC

                          ____________ III ____________
    TyrGlyGluAlaLeuCysLysValLeuIleGlyPhePheTyrGlyAsnMetTyrCysSer
    TACGGGGAGGCCCTGTGCAAGGTGCTCATTGGCTTTTTCTATGGTAACATGTATTGCTCC 480

                ____________
161 IleLeuPheMetThrCysLeuSerValGlnArgTyrTrpValIleValAsnProMetGly
    ATCCTCTTCATGACCTGCCTCAGCGTGCAGAGGTACTGGGTGATCGTGAACCCCATGGGA

                          ____________ IV ____________
    HisProArgLysLysAlaAsnIleAlaValGlyValSerLeuAlaIleTrpLeuLeuIle
    CACCCCAGGAAGAAGGCAAACATCGCCGTTGGCGTCTCCTTGGCAATCTGGCTCCTGATT 600
```

FIG.1A

```
                                                                    ▼
201 PheLeuValThrIleProLeuTyrValMetLysGlnThrIleTyrIleProAlaLeuAsn
    TTTCTGGTCACCATCCCTTTGTATGTCATGAAGCAGACCATCTACATTCCAGCATTGAAC

    IleThrThrCysHisAspValLeuProGluGluValLeuValGlyAsnMetPheAsnTyr
    ATCACCACCTGTCACGATGTGCTGCCTGAGGAGGTATTGGTGGGGGACATGTTCAATTAC 720

                                          V
241 PheLeuSerLeuAlaIleGlyValPheLeuPheProAlaLeuLeuThrAlaSerAlaTyr
    TTCCTCTCACTGGCCATTGGAGTCTTCCTGTTCCCGGCCCTCCTTACTGCATCTGCCTAC

    ValLeuMetIleLysThrLeuArgSerSerAlaMetAspGluHisSerGluLysLysArg
    GTGCTCATGATCAAGACGCTCCGCTCTTCTGCTATGGATGAACACTCAGAGAACAAAAGG 840

                                                VI
281 GlnArgAlaIleArgLeuIleIleThrValLeuAlaMetTyrPheIleCysPheAlaPro
    CAGAGGGCTATCCGACTCATCATCACCGTGCTGGCCATGTACTTCATCTGCTTTCGTCCT

    SerAsnLeuLeuLeuValValHisTyrPheLeuIleLysThrGlnArgGlnSerHisVal
    AGCAACCTTCTGCTCGTAGTGCATTATTTCCTAATCAAAACCCAGAGGCAGAGCCACGTC 960

                                                VII
321 TyrAlaLeuTyrLeuValAlaLeuCysLeuSerThrLeuAsnSerCysIleAspProPhe
    TACGCCCTCTACCTTGTCGCCCTCTGCCTGTCGACCCTCAACAGCTGCATAGACCCCTTT

    ValTyrTyrPheValSerLysAspPheArgAspHisAlaArgAsnAlaLeuLeuCysArg
    GTCTATTACTTTGTCTCAAAAGATTTCAGGGATCACGCCAGAAACGCGCTCCTCTGCCGA 1080

361 SerValArgThrValAsnArgMetGlnIleSerLeuSerSerAsnLysPheSerArgLys
    AGTGTCCGCACTGTGAATCGCATGCAAATCTCGCTCAGCTCCAACAAGTTCTCCAGGAAG
    GATGTCAAGCCTGCTTGATGATGATGATGATGATGGTGTGTGTGTG                 1246

    SerGlySerTyrSerSerSerSerThrSerValLysThrSerTyr
    TCCGGCTCCTACTCTTCAAGCTCAACCAGTGTTAAAACCTCCTACTGAGCTGTACCTGAG 1200
```

FIG. IB

```
                CGCTCCAGGCCTGGGTGACAGCGAGACCCTGTCTCATAAATTAAAAAATGAATAA

                                                SP
MetAsnValLeuSerPheGluGlnThrSerValThrAlaGluThrPheIleSerValMet
ATGAATGTACTTTCATTTGAACAAACCAGTGTTACTGCTGAAACATTTATTTCTGTAATG

                                 ▼                  ▽
ThrLeuValPheLeuSerCysThrGlyThrAsnArgSerSerLysGlyArgSerLeuIle   -1
ACCCTTGTCTTCCTTTCTTGTACAGGAACCAATAGATCCTCTAAAGGAAGAAGCCTTATT  120

GlyLysValAspGlyThrSerHisValThrGlyLysGlyValThrValGluThrValPhe
GGTAAGGTTGATGGCACATCCCACGTCACTGGAAAAGGAGTTACAGTTGAAACAGTCTTT

SerValAspGluPheSerAlaSerValLeuThrGlyLysLeuThrThrValPheLeuPro
TCTGTGGATGAGTTTTCTGCATCTGTCCTCACTGGAAAACTGACCACTGTCTTCCTTCCA  240

                                                I
IleValTyrThrIleValPheValValGlyLeuProSerAsnGlyMetAlaLeuTrpVal
ATTGTCTACACAATTGTGTTTGTGGTGGGTTTGCCAAGTAACGGCATGGCCCTGTGGGTC

PheLeuPheArgThrLysLysLysHisProAlaValIleTyrMetAlaAsnLeuAlaLeu
TTTCTTTTCCGAACTAAGAAGAAGCACCCTGCTGTGATTTACATGGCCAATCTGGCCTTG  360

          II
AlaAspLeuLeuSerValIleTrpPheProLeuLysIleAlaTyrHisIleHisGlyAsn
GCTGACCTCCTCTCTGTCATCTGGTTCCCCTTGAAGATTGCCTATCACATACATGGCAAC

AsnTrpIleTyrGlyGluAlaLeuCysAsnValLeuIleGlyPhePheTyrGlyAsnMet
AACTGGATTTATGGGGAAGCTCTTTGTAATGTGCTTATTGGCTTTTTCTATGGCAACATG  480

          III
TyrCysSerIleLeuPheMetThrCysLeuSerValGlnArgTyrTrpValIleValAsn
TACTGTTCCATTCTCTTCATGACCTGCCTCAGTGTGCAGAGGTATTGGGTCATCGTGAAC

ProMetGlyHisSerArgLysLysAlaAsnIleAlaIleGlyIleSerLeuAlaIleTrp
CCCATGGGGCACTCCAGGAAGAAGGCAAACATTGCCATTGGCATCTCCCTGGCAATATGG  600
```

FIG. 2A

IV

LeuLeuIleLeuLeuValThrIleProLeuTyrValValLysGlnThrIlePheIlePro
CTGCTGATTCTGCTGGTCACCATCCCTTTGTATGTCGTGAAGCAGACCATCTTCATTCCT

AlaLeuAsnIleThrThrCysHisAspValLeuProGluGlnLeuLeuValGlyAspMet
GCCCTGAACATCACGACCTGTCATGATGTTTTGCCTGAGCAGCTCTTGGTGGGAGACATG 720

V

PheAsnTyrPheLeuSerLeuAlaIleGlyValPheLeuPheProAlaPheLeuThrAla
TTCAATTACTTCCTCTCTCTGGCCATTGGGGTCTTTCTGTTCCCAGCCTTCCTCACAGCC

SerAlaTyrValLeuMetIleArgMetLeuArgSerSerAlaMetAspGluAsnSerGlu
TCTGCCTATGTGCTGATGATCAGAATGCTGCGATCTTCTGCCATGGATGAAAACTCAGAG 840

VI

LysLysArgLysArgAlaIleLysLeuIleValThrValLeuAlaMetTyrLeuIleCys
AAGAAAAGGAAGAGGGCCATCAAACTCATTGTCACTGTCCTGGCCATGTACCTGATCTGC

PheThrProSerAsnLeuLeuLeuValValHisTyrPheLeuIleLysSerGlnGlyGln
TTCACTCCTAGTAACCTTCTGCTTGTGGTGCATTATTTTCTGATTAAGAGCCAGGGCCAG 960

VII

SerHisValTyrAlaLeuTyrIleValAlaLeuCysLeuSerThrLeuAsnSerCysIle
AGCCATGTCTATGCCCTGTACATTGTAGCCCTCTGCCTCTCTACCCTTAACAGCTGCATC

AspProPheValTyrTyrPheValSerHisAspPheArgAspHisAlaLysAsnAlaLeu
GACCCCTTTGTCTATTACTTTGTTTCACATGATTTCAGGGATCATGCAAAGAACGCTCTC 1080

LeuCysArgSerValArgThrValLysGlnMetGlnValSerLeuThrSerLysLysHis
CTTTGCCGAAGTGTCCGCACTGTAAAGCAGATGCAAGTATCCCTCACCTCAAAGAAACAC

SerArgLysSerSerSerTyrSerSerSerSerThrThrValLysThrSerTyr *
TCCAGGAAATCCAGCTCTTACTCTTCAAGTTCAACCACTGTTAAGACCTCCTATTGAGTT 1200

FIG. 2B

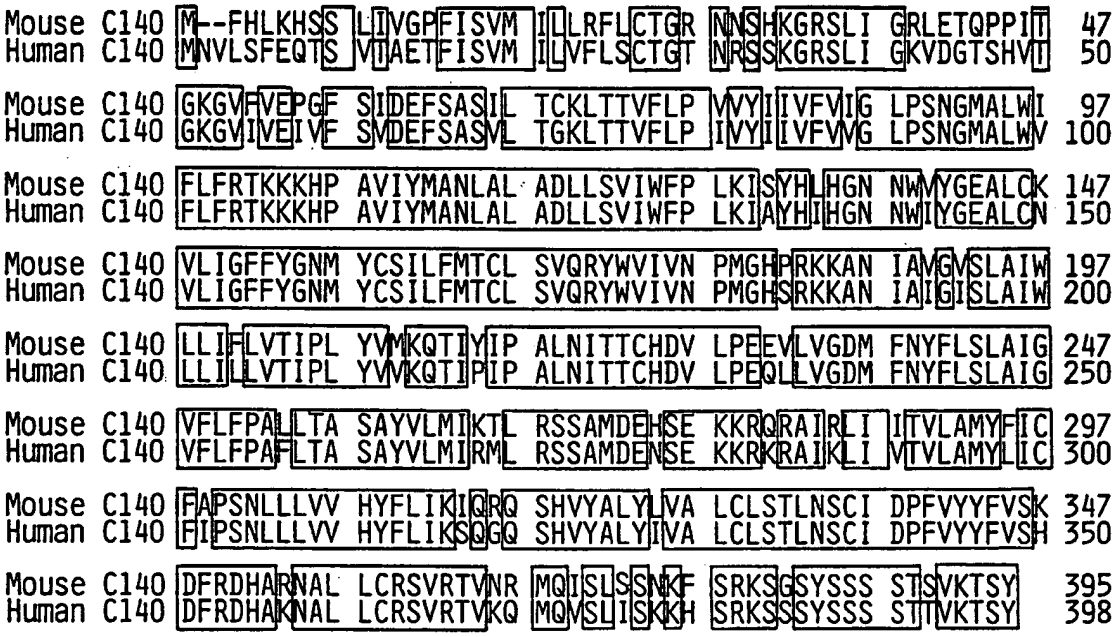
Mouse C140 M--FHLKHSS LIIVGPFISVM ILLRFLCTGR NNSHKGRSLI GRLETQPPIT 47
Human C140 MNVLSFEQTS VTIAETFISVM ILVFLSCTGT NRSSKGRSLI GKVDGTSHVT 50
Mouse C140 GKGVFVEPGF SIDEFSASIL TCKLTTVFLP VVYIIVFVIG LPSNGMALWI 97
Human C140 GKGVIVEIVF SMDEFSASVL TGKLTTVFLP IVYIIVFVVG LPSNGMALWV 100
Mouse C140 FLFRTKKKHP AVIYMANLAL ADLLSVIWFP LKISYHLHGN NWMYGEALCK 147
Human C140 FLFRTKKKHP AVIYMANLAL ADLLSVIWFP LKIAYHIHGN NWIYGEALCN 150
Mouse C140 VLIGFFYGNM YCSILFMTCL SVQRYWVIVN PMGHPRKKAN IAMGVSLAIW 197
Human C140 VLIGFFYGNM YCSILFMTCL SVQRYWVIVN PMGHSRKKAN IAIGISLAIW 200
Mouse C140 LLIFLVTIPL YVMKQTIYIP ALNITTCHDV LPEEVLVGDM FNYFLSLAIG 247
Human C140 LLILLVTIPL YVMKQTIPIP ALNITTCHDV LPEQLLVGDM FNYFLSLAIG 250
Mouse C140 VFLFPALLTA SAYVLMIKTL RSSAMDEHSE KKRQRAIRLI ITVLAMYFIC 297
Human C140 VFLFPAFLTA SAYVLMIRML RSSAMDENSE KKRKRAIKLI VTVLAMYLIC 300
Mouse C140 FAPSNLLLVV HYFLIKIQRQ SHVYALYIVA LCLSTLNSCI DPFVYYFVSK 347
Human C140 FIPSNLLLVV HYFLIKSQGQ SHVYALYIVA LCLSTLNSCI DPFVYYFVSH 350
Mouse C140 DFRDHARNAL LCRSVRTVNR MQISLSSNKF SRKSGSYSSS STSVKTSY 395
Human C140 DFRDHAKNAL LCRSVRTVKQ MQVSLISKKH SRKSSSYSSS STTVKTSY 398

FIG.3

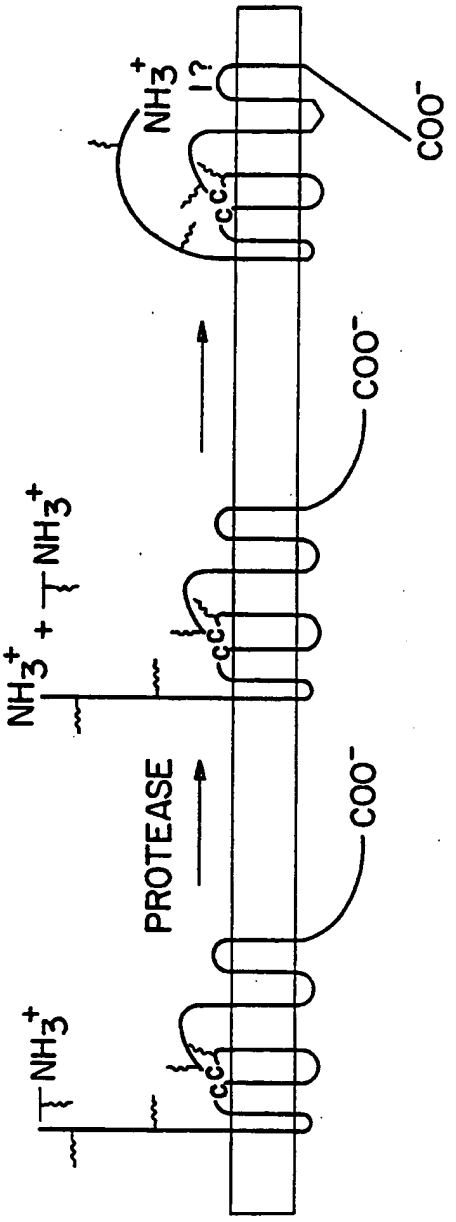

FIG. 4

```
                    SP                                  ▼
C140    MFHLKHSSLTVGPFISVMILLRFLCTGRNNSK-------GRSLIGRLETQP---------  44
HSTHRR  MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLDPRSFLLRNPNDKYEPEWEDEE  60

                                                          I
C140    ------------PITGKGVPVEPGFSIDEFSASILTGKLTTVFLPVVYIIVFVIGLPSN  91
HSTHRR  KNESGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWLTLFVPSVYTGVFVVSLPLN 120

                        II
C140    GMALWIFLFRTKKKHPAVIYMANLALADLLSVIWFPLKISYHLHGNNWVYGEALCKVLIG 151
HSTHRR  IMAIVVFILKMKVKKPAVVYMLHLATADVLFVSVLPFKISYYFSGSDWQFGSELCRFVTA 180

        III                                   IV
C140    FFYGNMYCSILFMTCLSVQRYWVIVNPM-GHPRKKANIAVGVSLAIWLLIFLVTIPLYVM 210
HSTHRR  AFYCNMYASILLMTVISIDRFLAVVYPMQSLSWRTLGRASFTCLAIWALAIAGVVPLVLK 240

                                          V
C140    KQTIYIPALNITTCHDVLPEEVLVGDMFNYFLSLAIGVFLFPALLTASAYVLMIKTLRSS 270
HSTHRR  EQTIQVPGLNITTCHDVLNETLLEGYYAYYFSAFSAVFFFVPLIISTVCYVSIIRCLSSS 300

                        VI
C140    AMDEHSEKKRQRAIRLIITVLAMYFICFAPSNLLLVVHY-FLIKTQRQSHVYALYLVALC 329
HSTHRR  AVANRSKKSR--ALFLSAAVFCIFIICFGPTNVLLIAHYSFLSHTSTTEAAYFAYLLCVC 358

        VII
C140    LSTLNSCIDPFVYYFVSKDFRDHARNALLCRSVRTVNRMQISLSSNKFSRKSGSYSSSST 389
HSTHRR  VSSISSCIDPLIYYYASSECQRYVYSILCCKESSDPSSYNSSGQLMASKMDTCSSNLNNS 418

C140    SVKTSY-                                                     395
HSTHRR  IYKKLLT                                                    426
```

FIG.5

8/16

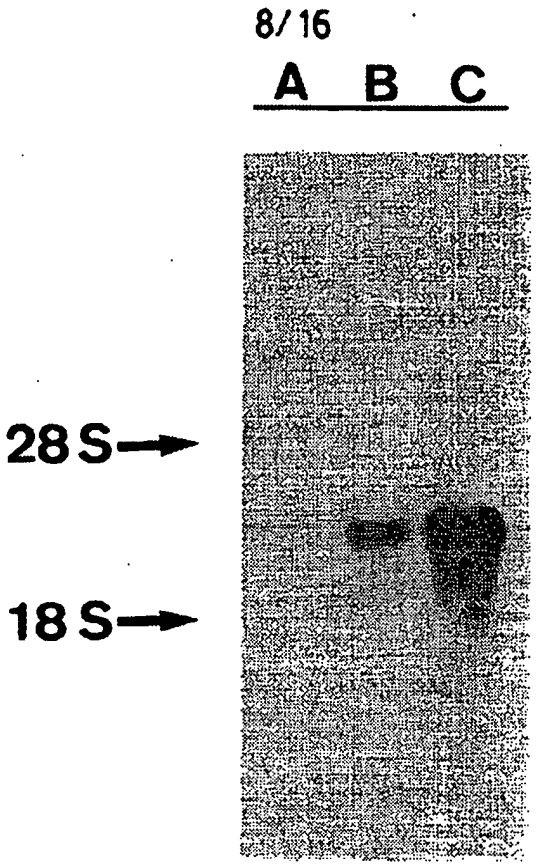

FIG.6

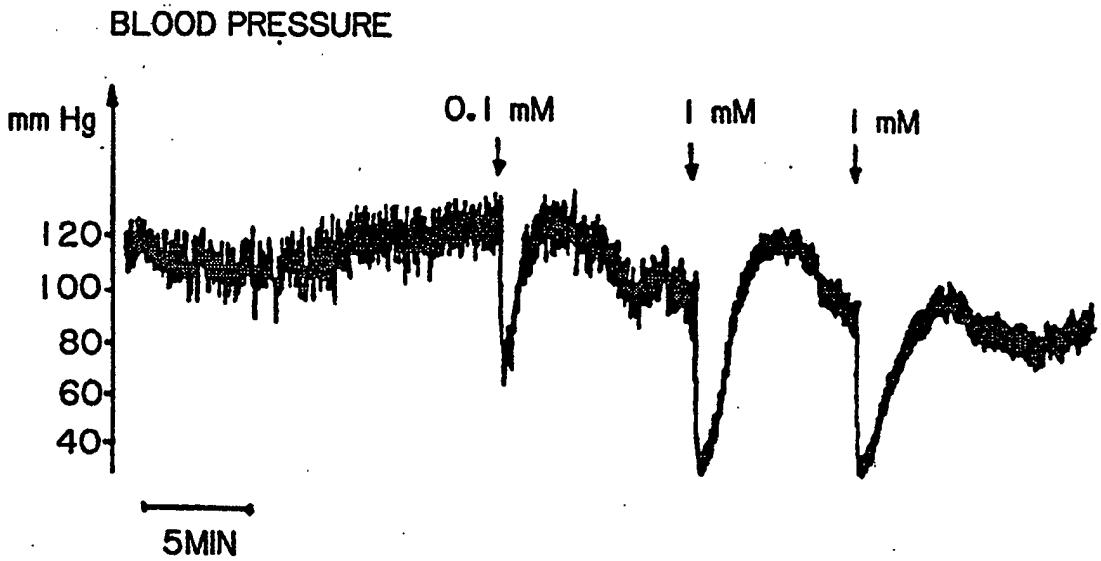

FIG.7

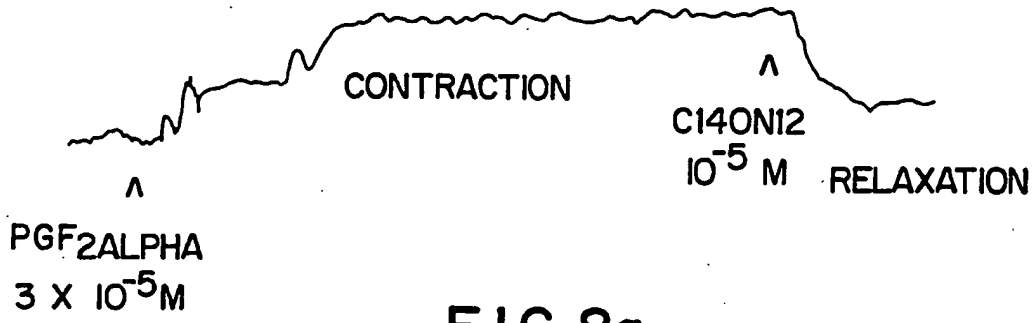
CONTRACTION
C14ON12
10^{-5} M
RELAXATION
PGF2ALPHA
3 X 10^{-5}M

FIG. 8a

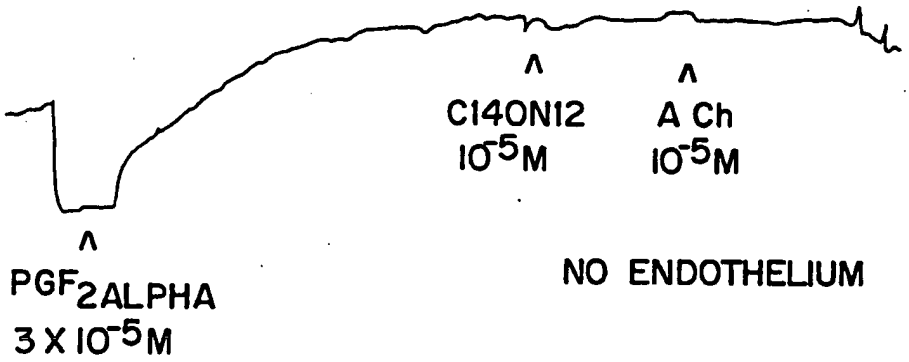
C14ON12
10^{-5}M
A Ch
10^{-5}M
NO ENDOTHELIUM
PGF2ALPHA
3 X 10^{-5}M

FIG. 8b

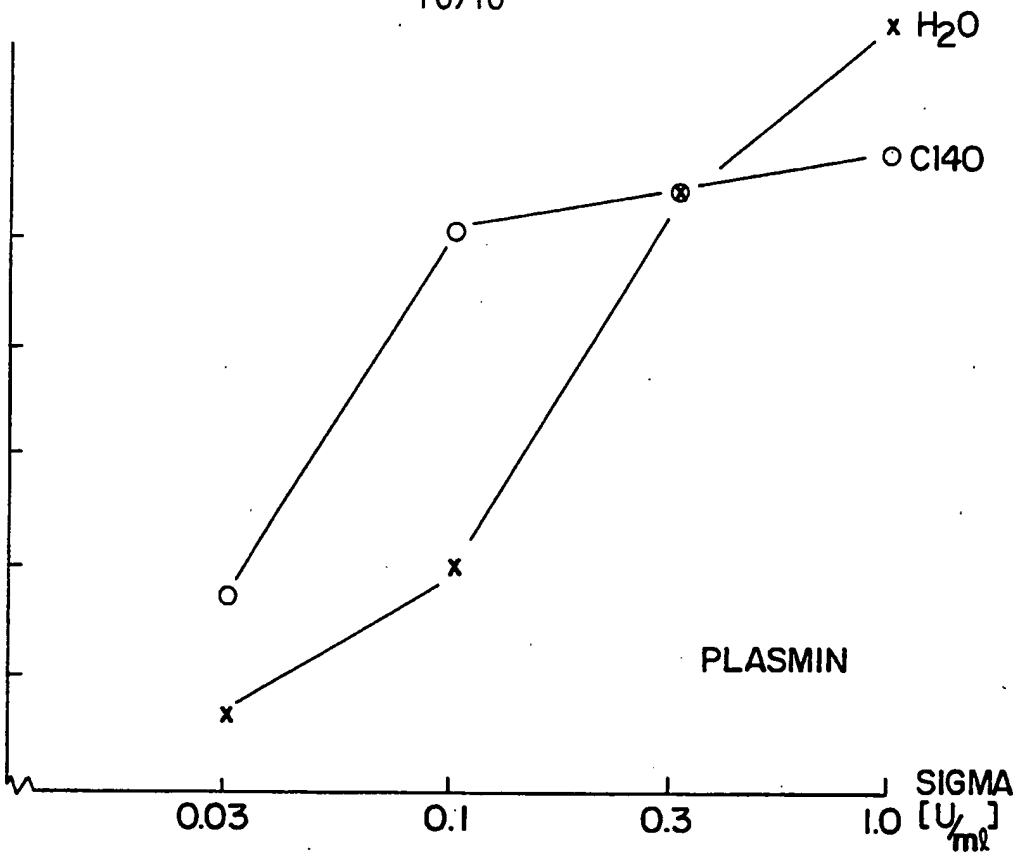
x H2O
C140
PLASMIN
0.03
0.1
0.3
1.0
SIGMA
[U/ml]

FIG.9a

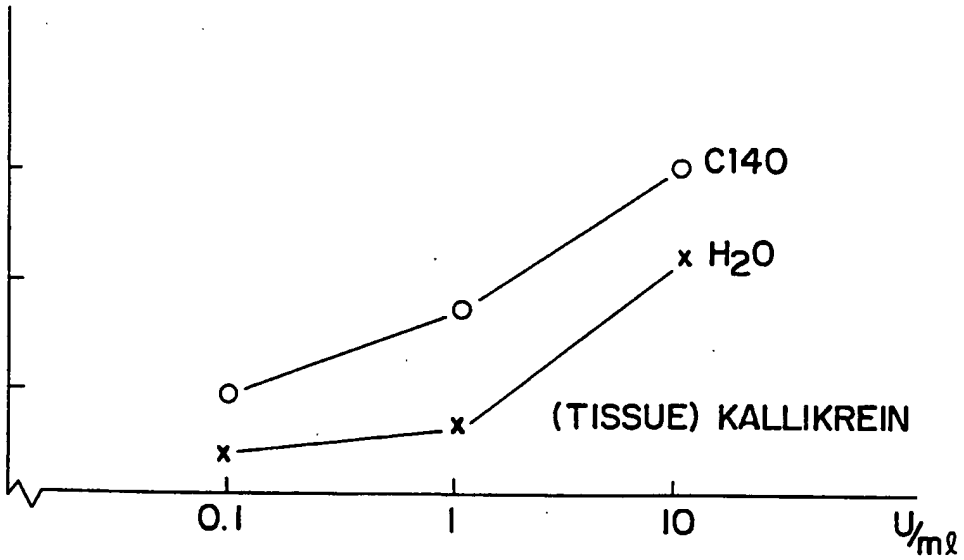
C140
x H2O
(TISSUE) KALLIKREIN
0.1
1
10
U/ml

FIG.9b

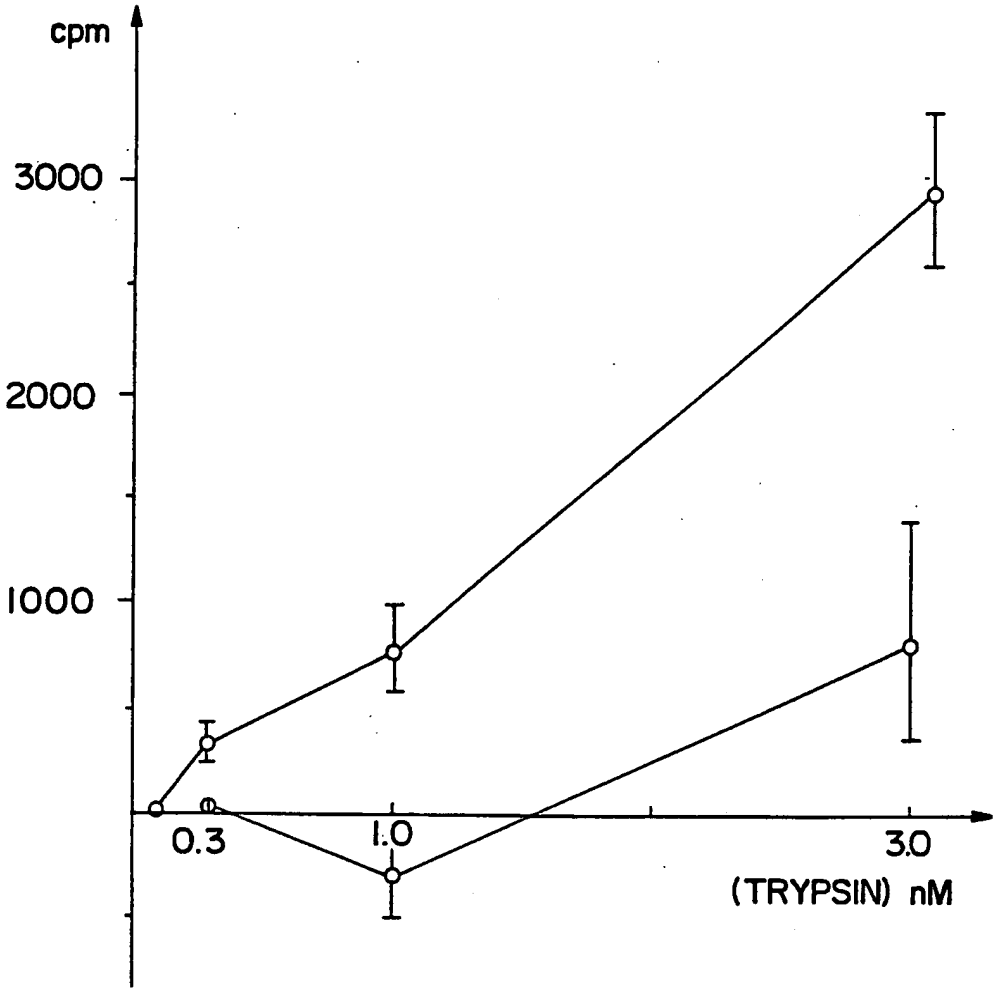

FIG.9c

```
CCCTGTGCTCAGAGTAGGGCTCCGAGTTTCGAACCACTGGTGGCGGATTGCCCGCCCGCC

CCACGTCCGGGGATGCGAAGTCTCAGCCTGGCGTGGCTGCTGGGAGGTATCACCCTTCTG
            M  R  S  L  S  L  A  W  L  L  G  G  I  T  L  L

GCGGCCTCGGTCTCCTGCAGCCGGACCGAGAACCTTGCACCGGGACGCAACAACAGTAAA
A  A  S  V  S  C  S  R  T  E  N  L  A  P  G  R  N  N  S  K

GGAAGAAGTCTTATTGGCAGATTAGAAACCCAGCCTCCAATCACTGGGAAAGGGGTTCCG
G  R  S  L  I  G  R  L  E  T  Q  P  P  I  T  G  K  G  V  P

GTAGAACCAGGCTTTTCCATCGATGAGTTCTCTGCGTCCATCCTCACCGGGAAGCTGACC
V  E  P  G  F  S  I  D  E  F  S  A  S  I  L  T  G  K  L  T

ACGGTCTTTCTTCCGGTCGTCTACATTATTGTGTTTGTGATTGGTTTGCCCAGTAATGGC
T  V  F  L  P  V  V  Y  I  I  V  F  V  I  G  L  P  S  N  G

ATGGCCCTCTGGATCTTCCTTTTCCGAACGAAGAAGAAACACCCCGCCGTGATTTACATG
M  A  L  W  I  F  L  F  R  T  K  K  K  H  P  A  V  I  Y  M

GCCAACCTGGCCTTGGCCGACCTCCTCTCTGTCATCTGGTTCCCCCTGAAGATCTCCTAC
A  N  L  A  L  A  D  L  L  S  V  I  W  F  P  L  K  I  S  Y

CACCTACATGGCAACAACTGGGTCTACGGGGAGGCCCTGTGCAAGGTGCTCATTGGCTTT
H  L  H  G  N  N  W  V  Y  G  E  A  L  C  K  V  L  I  G  F

TTCTATGGTAACATGTATTGCTCCATCCTCTTCATGACCTGCCTCAGCGTGCAGAGGTAC
F  Y  G  N  M  Y  C  S  I  L  F  M  T  C  L  S  V  Q  R  Y

TGGGTGATCGTGAACCCCATGGGACACCCCAGGAAGAAGGCAAACATCGCCGTTGGCGTC
W  V  I  V  N  P  M  G  H  P  R  K  K  A  N  I  A  V  G  V

TCCTTGGCAATCTGGCTCCTGATTTTTCTGGTCACCATCCCTTTGTATGTCATGAAGCAG
S  L  A  I  W  L  L  I  F  L  V  T  P  I  L  Y  V  M  K  Q

ACCATCTACATTCCAGCATTGAACATCACCACCTGTCACGATGTGCTGCCTGAGGAGGTA
T  I  Y  I  P  A  L  N  I  T  T  C  H  D  V  L  P  E  E  V

TTGGTGGGGGACATGTTCAATTACTTCCTCTCACTGGCCATTGGAGTCTTCCTGTTCCCG
L  V  G  D  M  F  N  Y  F  L  S  L  A  I  G  V  F  L  F  P

GCCCTCCTTACTGCATCTGCCTACGTGCTCATGATCAAGACGCTCCGCTCTTCTGCTATG
A  L  L  T  A  S  A  Y  V  L  M  I  K  T  L  R  S  S  A  M

GATGAACACTCAGAGAAGAAAAGGCAGAGGGCTATCCGACTCATCATCACCGTGCTGGCC
D  E  H  S  E  K  K  R  Q  R  A  I  R  L  I  I  T  V  L  A

ATGTACTTCATCTGCTTTGCTCCTAGCAACCTTCTGCTCGTAGTGCATTATTTCCTAATC
M  Y  F  I  C  F  A  P  S  N  L  L  L  V  V  H  Y  F  L  I

AAAACCCAGAGGCAGAGCCACGTCTACGCCCTCTACCTTGTCGCCCTCTGCCTGTCGACC
K  T  Q  R  Q  S  H  V  Y  A  L  Y  L  V  A  L  C  L  S  T

CTCAACAGCTGCATAGACCCCTTTGTCTATTACTTTGTCTCAAAAGATTTCAGGGATCAC
L  N  S  C  I  D  P  F  V  Y  Y  F  V  S  K  D  F  R  D  H
```

FIG. 10A

GCCAGAAACGCGCTCCTCTGCCGAAGTGTCCGCACTGTGAATCGCATGCAAATCTCGCTC
A R N A L L C R S V R T V N R M Q I S L

AGCTCCAACAAGTTCTCCAGGAAGTCCGGCTCCTACTCTTCAAGCTCAACCAGTGTTAAA
S S N K F S R K S G S Y S S S S T S V K

ACCTCCTACTGAGCTGTACCTGAGGATGTCAAGCCTGCTTGATGATGATGATGATGATGG
T S Y

TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGCACCCGTGTGTGAGTGCGTG

GTAGGGATACACCAACATGGATGGGGCTGTCATTTCCTATCCAAGCTGTCTGTCTCTGCA

CCAATCACAAGCATGCAGCTCTCCCCAGGATTGACAGAAGCCTCCTCCTTTGCATGAGAA

CAGTCTTCCACTCTGATGAAAAGCATCAGTATCAGAAACTGAAACGAACTGAGAGGAGCT

TGTTTTGTGAAAGTGAAGAGAAGATGGAGGGTCAGTGACTTGCAAAAAAAACCAACCAAA

CAAAAACTACACCTGGCAAGAAGGCTAAGACTCTCTGAAATGCTTCCCTTTTCCATCTGG

AGTTCGTCTCGGCCTTGTTCAGGACCTGAGGCCCTGGTAGAGCTTCAGTCCAGTTGATTG

ACTTTACAGACTTGAGAGAGGAGTGAATGAGGAGTGAATGAGGCTCCTGGCGGCATCCTA

ACCGGCTAACAGTGGCCTTGCTGGACAATAGGATTCAGATGGCTGGAGTTACATTCTCAC

ACCATTTCATCAGAACTATTGGGGATCTTGATCAATGTGCAGGTCCCTTAGCGTCAGTAA

CCCTGGGAGCTCAGACACGATGGGGGTGAGGGTGGGGGTGGGGGTGGGGGTGAGGCTCTA

CAAACCTTAGTGATGACTGCAGACACAGAACCATGGAGCTGAGCCTGCTTCTGCTTGCCA

GGGCACCACTGTAATGTTGGCAAAGAAAAACCAACAGCAGTGTTTTGAGCCTCTTTTTTT

GGTCAGTTTATGATGAATTTGCCTATTGGTTTATTGGGATTTTCAGTTCCTTTATTACTT

TGTTGTAATTTTGTGTGTTTATTAGTCAAGAAAAAGAAGATGAGGCTCTTAAAAATGTAA

ATAAAATTTTTGGTTTTTTGGTTTTTTAACTTGGGCCAACTACAAATACTGCTTAGGTTT

TTTTCTAACTTAATTGTTAACTACATCATGTGAACTTAAGACATTTTCATGATAAAGCAT

TACTGTAGTGTCAGTTTTCCCTCATCCTCGATCATAGTCCTTCCCGTGAAGCAGGGCCCT

TCCCCTCCCCCCCCTTTGCCGTTTCCCTCCCCACCAGATAGTCCCCCTGTCTGCTTTAAC

CTACCAGTTAGTATTTTATAAAAACAGATCATTGGAATATTTATTATCAGTTTTGTTCAC

TTGTTATCAGTTTTGTTCACTAATTTGTCCAATAATGGAATTAACGTCTTCTCATCTGTT

TGAGGAAGATCTGAAACAAGGGGCCATTGCAGGAGTACATGGCTCCAGGCTTACTTTATA

TACTGCCTGTATTTGTGGCTTTAAAAAAATGACCTTGTTATATGAATGCTTTATAAATAA

ATAATGCATGAACTTTAAAAAAAAAAAAAAA

FIG. 10B

```
         10         20         30         40         50         60
12345678901234567890123456789012345678901234567890123456789012345678
CAAAGAATTGTAATACGACTCACTATAGGGCGAATTCGGATCCAGGAGGATGCGGAGCCC
                                                    MetArgSerPr

         70         80         90        100        110        120
12345678901234567890123456789012345678901234567890123456789012345678
CAGCGCGGCGTGGCTGCTGGGGGCCGCCATCCTGCTAGCAGCCTCTCTCTCCTGCAGTGG      120
oSerAlaAlaTrpLeuLeuGlyAlaAlaIleLeuLeuAlaAlaSerLeuSerCysSerGl

CACCATCCAAGGAACCAATAGATCCTCTAAAGGAAGAAGCCTTATTGGTAAGGTTGATGG
yThrIleGlnGlyThrAsnArgSerSerLysGlyArgSerLeuIleGlyLysValAspGl

CACATCCCACGTCACTGGAAAAGGAGTTACAGTTGAAACAGTCTTTTCTGTGGATGAGTT      240
yThrSerHisValThrGlyLysGlyValThrValGluThrValPheSerValAspGluPh

TTCTGCATCTGTCCTCGCTGGAAAACTGACCACTGTCTTCCTTCCAATTGTCTACACAAT
eSerAlaSerValLeuAlaGlyLysLeuThrThrValPheLeuProIleValTyrThrIl

TGTGTTTGCGGTGGGTTTGCCAAGTAACGGCATGGCCCTATGGGTCTTTCTTTTCCGAAC      360
eValPheAlaValGlyLeuProSerAsnGlyMetAlaLeuTrpValPheLeuPheArgTh

TAAGAAGAAGCACCCTGCTGTGATTTACATGGCCAATCTGGCCTTGGCTGACCTCCTCTC
rLysLysLysHisProAlaValIleTyrMetAlaAsnLeuAlaLeuAlaAspLeuLeuSe

TGTCATCTGGTTCCCCTTGAAGATTGCCTATCACATACATGGCAACAACTGGATTTATGG      480
rValIleTrpPheProLeuLysIleAlaTyrHisIleHisGlyAsnAsnTrpIleTyrGl

GGAAGCTCTTTGTAATGTGCTTATTGGCTTTTTCTATCGCAACATGTACTGTTCCATTCT
yGluAlaLeuCysAsnValLeuIleGlyPhePheTyrGlyAsnMetTyrCysSerIleLu

CTTCATGACCTGCCTCAGTGTGCAGAGGTATTGGGTCATCGTGAACCCCATGGGGCACTC      600
uPheMetThrCysLeuSerValGlnArgTyrTrpValIleValAsnProMetGlyHisSe

CAGGAAGAAGGCAAACATTGCCATTGGCATCTCCCTGGCAATATGGCTGCTGACTCTGCT
rArgLysLysAlaAsnIleAlaIleGlyIleSerLeuAlaIleTrpLeuLeuThrLeuLe

GGTCACCATCCCTTTGTATGTCGTGAAGCAGACCATCTTCATTCCTGCCCTGAACATCAC      720
uValThrIleProLeuTyrValValLysGlnThrIlePheIleProAlaLeuAsnIleTh
```

FIG.IIA

GACCTGTCATGATGTTTTGCCTGAGCAGCTCTTGGTGGGAGACATGTTCAATTACTTCCT
rThrCysHisAspValLeuProGluGlnLeuLeuValGlyAspMetPheAsnTyrPheLe

CTCTCTGGCCATTGGGGTCTTTCTGTTCCCAGCCTTCCTCACAGCCTCTGCCTATGTGCT 840
uSerLeuAlaIleGlyValPheLeuPheProAlaPheLeuThrAlaSerAlaTyrValLe

GATGATCAGAATGCTGCGATCTTCTGCCATGGATGAAAACTCAGAGAAGAAAAGGAAGAG
uMetIleArgMetLeuArgSerSerAlaMetAspGluAsnSerGluLysLysArgLysAr

GGCCATCAAACTCATTGTCACTGTCCTGGGCATGTACCTGATCTGCTTCACTCCTAGTAA 960
gAlaIleLysLeuIleValThrValLeuGlyMetTyrLeuIleCysPheThrProSerAs

CCTTCTGCTTGTGGTGCATTATTTTCTGATTAAGAGCCAGGGCCAGAGCCATGTCTATGC
nLeuLeuLeuValValHisTyrPheLeuIleLysSerGlnGlyGlnSerHisValTyrAl

CCTGTACATTGTAGCCCTCTGCCTCTCTACCCTTAACAGCTGCATCGACCCCTTTGTCTA 1080
aLeuTyrIleValAlaLeuCysLeuSerThrLeuAsnSerCysIleAspProPheValTy

TTACTTTGTTTCACATGATTTCAGGGATCATGCAAAGAACGCTCTCCTTTGCCGAAGTGT
rTyrPheValSerHisAspPheArgAspHisAlaLysAsnAlaLeuLeuCysArgSerVa

CCGCACTGTAAAGCAGATGCAAGTACCCCTCACCTCAAAGAAACACTCCAGGAAATCCAG 1200
lArgThrValLysGlnMetGlnValProLeuThrSerLysLysHisSerArgLysSerSe

CTCTTACTCTTCAAGTTCAACCACTGTTAAGACCTCCTATTGAGTTTTCCAGGTCCTCAG
rSerTyrSerSerSerSerThrThrValLysThrSerTyr

ATGGGAATTGCACAGTAGGATGTGGAACCTGTTTAATGTTATGAGGACGTGTCTGTTATT 1320

TCCGGATCCAGATCTTATTAAAGCAGAACTTGTTTATTGCAGCTTATAATGGTTACAAAT

AAAGCAATAGCATCACAAATTTCACAAATAAAGC 1414

FIG.11B

FIG.12

1 2 3 4 5 6 7 8 9 10 11 12 13 14

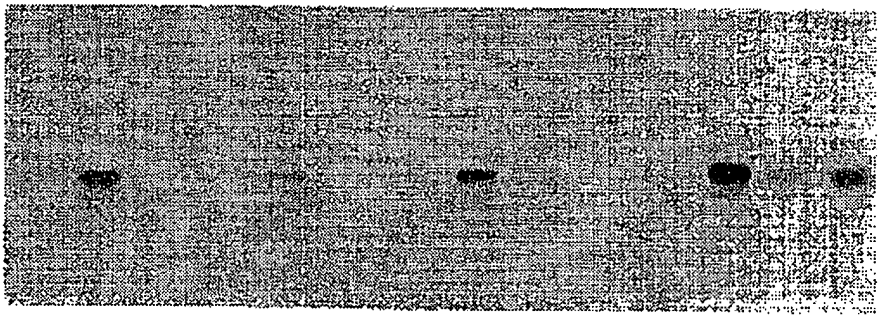

FIG.13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08536

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/00, 21/04; C07K 7/00, 7/06, 7/08, 15/00
US CL :435/7.1, 7.2, 7.21; 530/327, 328, 329; 536/23.1, 23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.2, 7.21; 530/327, 328, 329; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Biosis, Medline, Biotech
Search terms: C140, receptor, antagonist, agonist,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,128,254 (SIBLEY ET AL.) 07 July 1992, see entire document.	1-21
A	The Journal of Biological Chemistry, Volume 267, Number 19, issued 05 July 1992, Scarborough et al., "Tethered Ligand Agonist Peptides", pages 13146-13149, see page 13147.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 SEPTEMBER 1994	Date of mailing of the international search report 27 OCT 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID GUZO Telephone No. (703) 308-0196

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